

Supplemental Material

Outdoor Formaldehyde and NO₂ Exposures and Markers of Genotoxicity in Children Living Near Chipboard Industries

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Table S1. Participation rates and number (%) of genotoxicity assays carried out for each sampling stratum, defined according to the distance of the children's houses (addresses collected by the baseline questionnaire) to the factories.

| Participation rates | ≥ 4 km from any wood factory (n=205) | <4 km from a small wood factory and ≥ 2 km from the chipboard industries (n=236) | <2 km from a chipboard industry (n=215) |
|--|---|---|--|
| N. children participating ^a | 135 (66%) | 146 (62%) | 132 (61%) |
| Micronuclei assays | 135 (66%) | 144 (61%) | 132 (61%) |
| Comet assays | 122 (60%) | 115 (49%) | 103 (48%) |

^aParticipation was defined as having the questionnaire answered and one (or both) the genotoxicity assays carried out.

Table S2: Main characteristics^a of the children who did and did not participate in the study.

| Characteristics | Participants^c (n=413) | Non participants (n=243) | p-value |
|-----------------------------------|---|-------------------------------------|----------------|
| Female sex | 182 (44.0) | 107 (44.1) | 0.999 |
| Age (y) ^b | 5.5±0.1 | 5.6±0.1 | 0.408 |
| Foreign nationality | 37 (9.0) | 43 (18.1) | 0.001 |
| Parents' education | | | 0.207 |
| <i>Primary school or less</i> | 11 (2.7) | 10 (4.3) | |
| <i>Secondary or professional</i> | 112 (27.6) | 75 (32.3) | |
| <i>High school</i> | 216 (53.3) | 120 (51.7) | |
| <i>University</i> | 66 (16.3) | 27 (11.6) | |
| Smoking parents | 142 (35.8) | 112 (48.9) | 0.001 |
| Exposure to tobacco smoke at home | 64 (15.7) | 47 (20.1) | 0.179 |
| High residential traffic level | 242 (59.2) | 152 (64.7) | 0.179 |

^aInformation was obtained from the baseline questionnaire. N (%) reported, unless stated otherwise. ^bAge in December 2006; mean ± SD reported. ^cParticipation was defined as having the questionnaire answered and one (or both) the genotoxicity assays carried out.

Table S3: Descriptive statistics on formaldehyde and NO₂ concentrations at the four 1-week measurement campaigns, and annual average concentrations.

| Statistics | Week 1 | Week 2 | Week 3 | Week 4 | Average^a |
|--|----------------|----------------|----------------|----------------|----------------------------|
| Starting date | 03/06/2010 | 29/06/2010 | 11/11/2010 | 16/12/2010 | |
| Formaldehyde, mean \pm SD ($\mu\text{g}/\text{m}^3$) | 2.3 \pm 0.5 | 3.4 \pm 0.7 | 1.6 \pm 0.4 | 2.6 \pm 0.6 | 2.5 \pm 0.3 |
| Formaldehyde, coefficient of variation | 0.21 | 0.22 | 0.26 | 0.24 | 0.14 |
| NO ₂ , Mean \pm SD ($\mu\text{g}/\text{m}^3$) | 12.1 \pm 4.5 | 13.9 \pm 4.2 | 14.9 \pm 5.8 | 22.8 \pm 9.2 | 16.0 \pm 3.5 |
| NO ₂ , coefficient of variation | 0.37 | 0.30 | 0.39 | 0.40 | 0.22 |

^aAdjusted for temporal variation to account for missing data (at monitoring sites with <4 measurements).

Table S4. Estimated associations (95% CI) of exposure to formaldehyde and NO₂ with markers of genotoxic damage: comparison between the main and sensitivity analyses.^a

| Outcome | Formaldehyde: main analysis | Formaldehyde: distance to chipboard industries < 4 km | Formaldehyde: additional adjustment for proxies of indoor air quality ^c | NO ₂ : main analysis | NO ₂ : distance to chipboard industries < 4 km | NO ₂ : additional adjustment for proxies of indoor air quality ^c |
|--|--------------------------------|---|---|------------------------------------|---|---|
| Comet assay^b | | | | | | |
| number of subjects with complete information | 310 | 118 | 229 | 310 | 118 | 229 |
| Tail intensity (TI): % change | 0.13 (0.03, 0.22)* | 0.19 (0.04, 0.34)* | 0.12 (0.01, 0.23)* | 0.06 (-0.05, 0.16) | 0.03 (-0.15, 0.21) | 0.09 (-0.03, 0.21) |
| Tail length (TL): µm change | -0.06 (-0.29, 0.17) | -0.10 (-0.46, 0.26) | -0.01 (-0.27, 0.25) | 0.10 (-0.14, 0.34) | 0.24 (-0.16, 0.64) | 0.04 (-0.24, 0.31) |
| Tail moment (TM) | 0.007 (0.001, 0.012)* | 0.012 (0.003, 0.020)** | 0.007 (0.001, 0.014)* | 0.004 (-0.002, 0.010) | 0.006 (-0.004, 0.016) | 0.005 (-0.002, 0.012) |
| Micronucleus assay | | | | | | |
| number of subjects with complete information | 374 | 155 | 273 | 374 | 155 | 273 |
| Binucleated cells (BN): % change | 0.02 (-0.05, 0.08) | -0.08 (-0.20, 0.03) | 0.02 (-0.05, 0.10) | 0.13 (0.07, 0.19)*** | 0.19 (0.07, 0.31)** | 0.15 (0.07, 0.22)*** |
| Micronuclei (MN): RR | 0.98 (0.91, 1.06) | 0.93 (0.82, 1.05) | 0.97 (0.89, 1.06) | 1.00 (0.93, 1.07) | 1.05 (0.92, 1.21) | 0.99 (0.91, 1.08) |
| Nuclear buds: RR | 1.12 (1.02, 1.23)* | 1.02 (0.89, 1.18) | 1.11 (1.01, 1.23)* | 1.16 (1.06, 1.26)** | 1.23 (1.06, 1.41)** | 1.17 (1.07, 1.28)** |

*p<0.05, **p<0.01, ***p<0.001

^aBoth the main and sensitivity analyses were adjusted for sex, age, nationality, parents’ education and smoking habits, exposure to tobacco smoke at home, average time of air refreshing, residential traffic level, presence of orthodontic appliance, DMFT score, person who collected the cell sample. Estimates are given for a 1-SD increase in exposure (0.20 µg/m³ and 2.13 µg/m³ for formaldehyde for NO₂, respectively). ^bWeighted for the number of cells examined (50 when available). ^cAlso adjusted for age of the house ≤5 years, non-solid (chipboard, plywood) wooden furniture in child’s bedroom, double glazed windows in child’s bedroom (information retrieved from the baseline questionnaire)

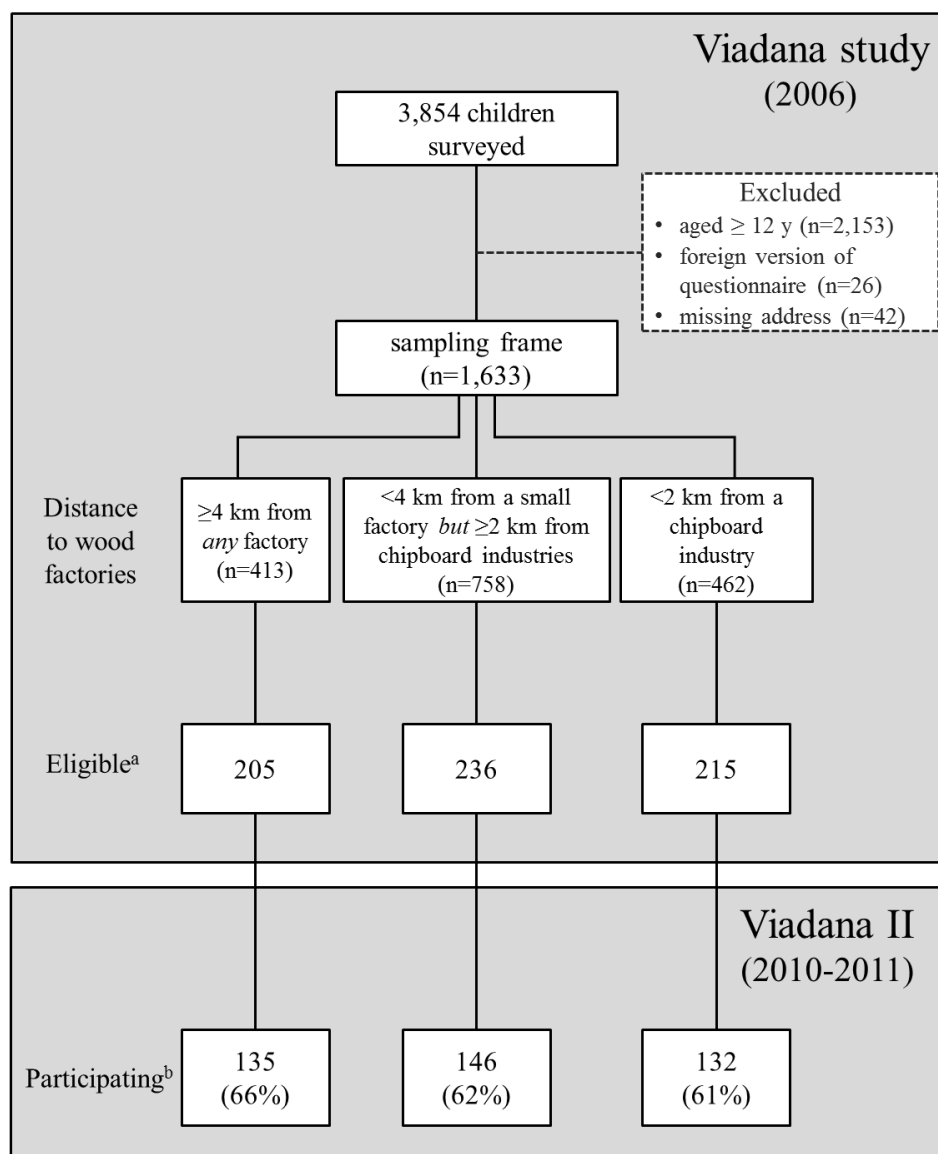


Figure S1: Selection of the children and participation in the Viadana II study. ^a250 children were randomly sampled from each sampling stratum. Then the children who had moved outside the Viadana district between 2006 and 2010 were excluded (n=94 in total). ^bParticipation was defined as having the questionnaire answered and one (or both) the genotoxicity assays carried out.

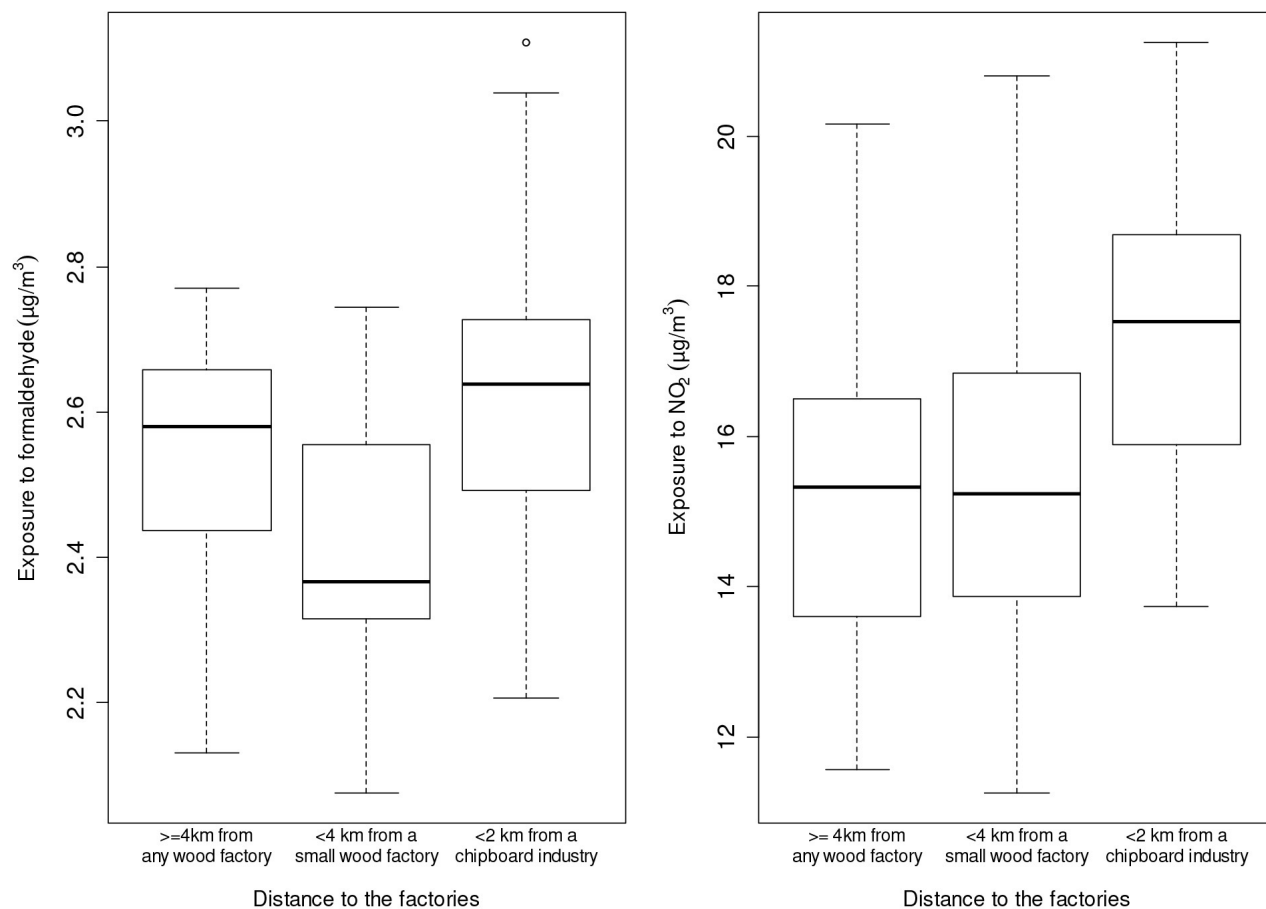


Figure S2: Box-plots representing the distribution of modelled exposure to formaldehyde and NO₂ by distance of children's houses to the factories.^a

^aBoxes extend from the 25th to the 75th percentile, horizontal bars represent the median, whiskers extend 1.5 times the length of the interquartile range (IQR) above and below the 75th and 25th percentiles, respectively, and outliers are represented as points.